Reference: *Biol. Bull.* **196:** 34–44. (February, 1999)

Differences in the Composition of Adhesive and Non-Adhesive Mucus From the Limpet *Lottia limatula*

ANDREW M. SMITH*, TONYA J. QUICK, AND RACHEL L. ST. PETER

Department of Biological Sciences, Butler University, Indianapolis, Indiana 46208

Abstract. The mucus used by the limpet *Lottia limatula* to form glue-like attachments was compared biochemically to the slippery mucus produced during other activities, such as suction adhesion. Colorimetric assays revealed the protein content of the adhesive mucus to be 2.1 times greater than that of the non-adhesive form, and the carbohydrate content to be 1.9 times greater. Both forms of mucus contained roughly six times as much protein as carbohydrate, and there was no difference in their inorganic elemental compositions. Quantitative analysis of the protein content by SDS-PAGE and a scanning densitometer revealed a similar protein composition in both forms of mucus; but three notable differences emerged. First, the overall difference in protein concentration was confirmed. In addition, there was a 118 kD protein that was common only in the adhesive mucus, and a 68 kD protein that occurred only in the non-adhesive mucus.

Introduction

Limpets are known for their ability to attach firmly to rocks in the wave-swept intertidal zone. Recently, Smith (1992) showed that many limpets alternate between two attachment mechanisms. At high tide on the California coast, most limpets use suction adhesion; when the tide goes out, most switch to a glue-like adhesion. Suction adhesion has been studied in limpets and cephalopod molluscs (Smith, 1991a,b; Smith *et al.*, 1993), but the glue-like adhesion of limpets has not been well studied. This adhesive forms a powerful, durable attachment, yet it is based on mucus rather than a solid cement.

This glue-like adhesive can be so strong that when one tries to remove limpets from a hard surface, the shell sometimes tears off before the adhesive fails. Smith (1992) estimated that the average tenacity, defined as force per unit area of attachment, of the adhesive secreted by four species of limpets from California is 230 kPa. Tenacities up to 518 kPa have been reported in other species (Branch and Marsh, 1978). Whether the forces reported by Branch and Marsh (1978) were due to suction or gluing was not made clear, but it is likely that the mechanism was gluing. This is because cavitation normally causes failure of suction adhesives at tenacities between 100 and 200 kPa at sea level (Smith, 1996).

The strength of this adhesive is surprising, because it is composed of mucus. Mucus is typically a dilute network of proteins and polysaccharides. These molecules take on an extended configuration that causes them to become entangled, thus forming a weak gel (Wainwright *et al.*, 1976). The viscoelasticity of such a gel can provide some mechanical strength (Denny, 1980, 1989; Grenon and Walker, 1980), but its stiffness is normally much lower than necessary for the tenacities limpets achieve (Smith, 1991b). Also, although high-viscosity materials can function as Stefan adhesives (Grenon and Walker, 1981), this mechanism has limitations that make it unlikely to be effective for invertebrates (see Discussion).

Mucous secretions are widely used as lubricants, not adhesives. For example, lubricating mucous gels line and protect the mammalian digestive, reproductive, and respiratory tracts (Silberberg and Meyer, 1982). In marine invertebrates, mucus typically forms a slippery coating that prevents bacteria and debris from accumulating on the body surface (Baier *et al.*, 1985), and keeps other organisms from adhering to it (Harrold, 1982). Thus, we would not expect typical mucous secretions to be good adhesives. Nevertheless, limpets do modify mucus so that it functions in this way.

The mechanical properties of mucus can be modified in

Received 3 September 1997; accepted 1 December 1998.

* To whom correspondence should be addressed. E-mail: ansmith@thomas.butler.edu

various ways (Chantler and deBruyne, 1977). For example, changing the pH or ion content of the water in the gel could cause the gel to shrink and thus stiffen. Alternatively, specific proteins or carbohydrates could be added to cross-link the gel-forming polymers. Finally, a different type of gel-forming polymer could be synthesized and secreted. Of course, some combination of these changes could occur. Moreover, all seem equally plausible: limpets can probably move specific ions across their pedal epithelia (Hermans, 1983), and there are a wide variety of glands secreting onto their pedal sole (Grenon and Walker, 1978). We propose that limpets secrete a specific form of mucus for lubrication while moving or using suction adhesion; then, when a stronger attachment is needed, the mucus is modified in one of the ways suggested above. To test this hypothesis, we compared the compositions of adhesive mucus and non-adhesive mucus, aiming to identify biochemical differences that might be correlated with the change in function.

Materials and Methods

Sample collection

Specimens of *Lottia limatula* (Carpenter) were purchased from Pacific Bio-Marine Labs, Inc. (Venice, CA, United States) and maintained in a recirculating marine aquarium at 18°C. Samples of mucus were collected primarily during the first 2 or 3 weeks after the arrival of the limpets, although the animals survived and continued to produce mucus for several months.

Adhesive mucus was collected from limpets that had been left undisturbed on a clean, glass aquarium wall for at least 24 h. In this situation, roughly one-third of the limpets would be attached using the glue-like mechanism (Smith, 1991b). These limpets were easily distinguished from those using suction; the limpets using glue stuck much more firmly, but their tenacity decreased dramatically once dislodged from their initial spot. After each limpet was dislodged, a thin film of mucus remained tightly adhered at the point of attachment. One could easily feel this patch of adhesive mucus, even underwater, where most of the samples were collected. This mucus was scraped off with a clean razor blade, forming distinct, irregular clumps that clung to the blade. The blade was gently shaken and tilted to remove excess water from the surface of the sample, which was then scraped into a microcentrifuge tube. Each tube contained between 10 and 50 mg of mucus collected from at least five limpets. The samples were pooled and stored at −70°C.

Non-adhesive mucus was collected as follows. Groups of 6 to 10 limpets were placed in a small plastic bag (roughly 1 liter) filled with seawater. The limpets rarely adhered to the bag and instead stayed active, often climbing on one another's shells, using suction. When removed from the bag after 4 to 8 h, many of the limpets had thin sheets and aggregations of mucus on the shell and across the sole of the foot. This mucus, which could be collected by gently rubbing it off of the shell or foot, was quite different from the adhesive mucus, which resisted fairly vigorous rubbing. Also, when compared side by side, the non-adhesive mucus seemed less elastic and dense than the adhesive mucus. After collection, excess water was removed in the same way as with the adhesive samples. The samples were then pooled and stored as above. Note that this collection method could not ensure that all the mucus was from the sole of the foot; some of it might have come from the hypobranchial glands, the mantle, or the sides of the foot. Nevertheless, this procedure provided a good sample of non-adhesive mucus.

Comparison of total protein and carbohydrate content, and inorganic content

The wet weights of three samples each of adhesive and non-adhesive mucus were measured on an analytical balance. They were then dehydrated in a SpeedVac, and their dry weight was measured.

The protein concentration of the mucus was measured using the Bradford assay (Bradford, 1976; Bio-Rad protein assay kit). Eleven samples of adhesive mucus and ten of non-adhesive mucus were assayed. Each sample of 10–40 mg of mucus was thawed, weighed, and placed in 200 μl of distilled, de-ionized water with 1% sodium dodecyl sulfate (SDS) and 1% dithiothreitol (DTT). The samples were dissolved by sonication for roughly 10 mins, then diluted so that the final SDS concentration was less than 0.1% to avoid interference with the Bradford assay. Several dilutions of bovine serum albumin with the same concentration of SDS and DTT were used as standards. In addition to the Bradford assay, many of the samples were also assayed at 230 and 280 nm (correcting for absorption at 260 nm as described by Boyer, 1993). Several samples were also dissolved in 1 *M* NaOH and assayed at 230 and 280 nm.

The carbohydrate concentration of the mucus was assayed using the orcinol-sulfuric acid method described by Kennedy and Pagliuca (1994). Seven samples of adhesive mucus and six samples of non-adhesive mucus were tested. The only differences from the published method were that orcinol was not recrystallized before use, and the assays were made at 510 nm, as suggested in the original method, rather than 420 nm, which Kennedy and Pagliuca suggest. Data were also collected at 420 nm, but the 510 absorbance gave more consistent results for the samples and the glucose standard.

The inorganic composition of five samples each of adhesive and non-adhesive mucus was also determined. Samples were dried in a SpeedVac and analyzed with a JEOL JSM-U3 scanning electron microscope outfitted with a Tracor Northern 5500 EDS (Energy Dispersive X-ray System). Samples were mounted on graphite backing and scanned at

20 kV. Of these 10 samples, 2 of each type were sonicated before drying to improve sample homogeneity. Sonication did not noticeably affect the results.

Comparison of protein composition using SDS-PAGE

The protein compositions of adhesive and non-adhesive mucus were compared by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Eleven samples of each type were compared, and they were trimmed with a razor blade so that similar wet weights were used. Nevertheless, on average, there was 1.13 ± 0.15 times (mean ± SD) as much of the non-adhesive form. Samples were dissolved as in the protein assays. They were run on discontinuous gels, based on the method of Laemmli (1970) and the detailed protocols of Hames (1990). The gel dimensions were 8 × 9 cm by 0.75 mm thick, and the total acrylamide concentration was 10%. Gels were stained with Coomassie blue R-250.

The SDS-PAGE results were averaged to eliminate variation due to proteins unrelated to adhesion. Each lane was scanned with a densitometer, measuring the absorbance at 580 nm. The results were recorded on a Macintosh computer using a MacADIOS II digitizer and Superscope II software (GW Instruments, Somerville, MA). The data for each lane were transferred to a spreadsheet and converted to a molecular weight scale. This conversion was based on calibration curves specific to each gel that were generated with a set of standard proteins (high molecular weight range, Sigma Scientific). The molecular weight values appear to be accurate to within 1 or 2 kD, except for the protein identified at 203 kD. This protein may be 10 to 20 kD larger. Once the data were converted, the staining intensity at each molecular weight was averaged for all 11 samples of each type of mucus.

In addition to the detailed comparison described above, other samples were tested under a variety of conditions. Different buffers were tested for their ability to solubilize the mucus. Factors that were varied included pH, salt content, and the presence and concentration of reagents such as urea, SDS, and 2-mercaptoethanol. Varying degrees of shear were also tested, ranging from sonication to mild rocking overnight. Gels with total acrylamide concentrations of 5% and 20% were also run. In a number of samples, the stacking gel was also scanned with the densitometer to quantify any material that was too large to enter the running gel.

Characterization of proteins

Several samples each of adhesive and non-adhesive mucus were stained for the presence of glycoproteins. The samples were dissolved in 0.125 *M* Tris-Cl (pH 6.8), 2% SDS, 5% 2-mercaptoethanol, and 10% glycerol and run on a 10% gel as described previously. The proteins were then transferred to a nitrocellulose membrane in a Mighty Small Transphor tank transfer unit (Hoefer Scientific Instruments), using the buffer of Towbin *et al.* (1979). Transfer conditions were 400 mA for 1 h. The membrane was stained transiently with Ponceau-S to ensure that all the proteins transferred. Glycoproteins were detected using a DIG Glycan Detection Kit (Boehringer Mannheim) following the instructions in the manual. Briefly, this protocol involved periodic acid oxidation followed by binding of digoxigenin to the oxidized sugars. These were marked by anti-digoxigenin antibodies conjugated to alkaline phosphatase.

Amino acid analysis was performed on some of the major proteins identified through SDS-PAGE. Samples of adhesive and non-adhesive mucus were solubilized as described in the previous paragraph and run on a 10% gel. Proteins were transferred to an immobilon-P membrane (Millipore), using 22.5 m*M* Tris-borate, 0.1 m*M* EDTA, 0.1% SDS and 25% methanol as a tank buffer. Running conditions were 400 mA for 3 h. The membrane was stained with Coomassie blue G-250 and destained with 40% methanol. Selected bands and a blank from outside the lanes were excised from the membrane and hydrolyzed in 6 *M* HCl with 10% phenol and 10% trifluoroacetic acid under vacuum at 150°C for 40 min. Amino acid compositions were then determined on a Beckman System 6300 Auto Analyzer. The amino acid composition of two whole, dried samples of adhesive mucus was also analyzed. The samples were dissolved in either ammonium hydroxide or trifluoroacetic acid, hydrolyzed for 22 h at 110°C with 6 *M* HCl and phenol, then run on a Beckman analyzer.

Samples were also analyzed by two-dimensional electrophoresis. Iso-electric focusing was used as the first dimension, followed by SDS-PAGE. Proteins were focused for 3 h in the presence of 9 *M* urea and 2% CHAPS. Iso-electric focusing was carried out using pH gradients from 3 to 10 and from 4 to 6. Two-dimensional gels were silver stained (Silver stain plus kit, Bio-Rad).

Results

Comparison of total protein and carbohydrate content

The overall polymer concentration of the adhesive secretion was roughly two times greater than that of the non-adhesive secretion (Fig. 1). On average, there was 2.1 times as much protein in the adhesive form as the non-adhesive form (Student's *t* test, $P < 0.001$). There was also 1.8 times as much carbohydrate in the adhesive form ($P < 0.001$). In both forms of mucus, protein made up a greater part of the secretion than carbohydrate. Assuming that protein and carbohydrates were the primary organic molecules present, protein made up 86% of the organic material in adhesive mucus and 85% in non-adhesive mucus.

The values for protein and carbohydrate concentration were slightly lower than what has been reported in other

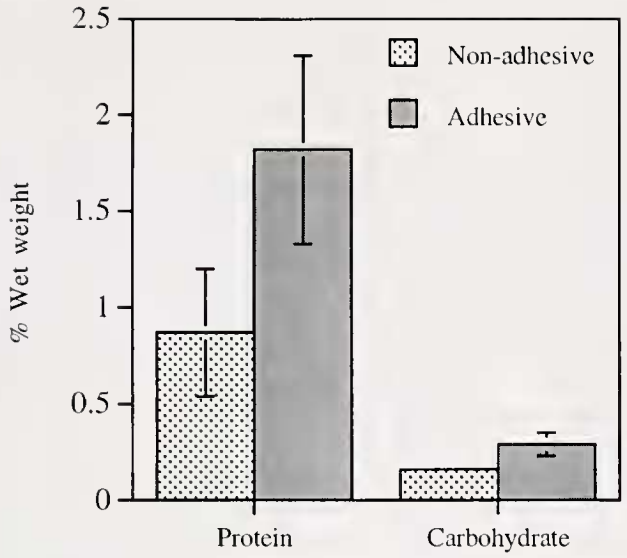

Figure 1. Comparison of the protein and carbohydrate contents of non-adhesive and adhesive mucus.

limpet species (typically about 3% protein and 1% carbohydrate; Davies *et al.,* 1990). This may be due partly to the resistance of limpet mucus to solubilization. Even under the conditions used in our experiments, some of the material failed to go into solution. Further tests were carried out using 4% SDS or 1 *M* NaOH to improve solubility. Since these reagents interfered with the Bradford assay, samples were assayed at 230 and 280 nm. The results of these tests support the finding that the adhesive form contains twice as much protein. The protein contents determined by these assays varied much more widely, but were typically somewhat higher than the values in Figure 1.

The water content of limpet pedal mucus was similar to values reported by Davies *et al.* (1990). The adhesive form of mucus contained 92.5% ± 3.3 water and the non-adhesive form contained 92.9% ± 1.3 water. Presumably, an additional 4% to 5% of the wet weight was composed of salts.

Comparison of inorganic content

There was no difference between the inorganic elemental compositions of adhesive and non-adhesive mucus (Fig. 2). The inorganic composition of both forms of mucus is similar to that of seawater, since mucus is typically more than 90% water. There was relatively more sulfur and phosphorus than in seawater, as expected for a solution containing organic molecules. Note that 3 of the 10 samples were omitted from the analysis because they appeared to be contaminated; more than 39% of the inorganic content was calcium in one non-adhesive sample and roughly 30% of the inorganic content was iron in the other two (one adhesive and one non-adhesive). In all but one of the other samples, calcium and iron each made up just 1% to 3% of the inorganic content. One of the adhesive samples in the analysis had 11% iron. This one sample accounted for the appearance of a slight difference in iron content between the two types of mucus. The presence of some heavy metals is probably also attributable to contamination. Mercury was found only in one sample, as was zinc.

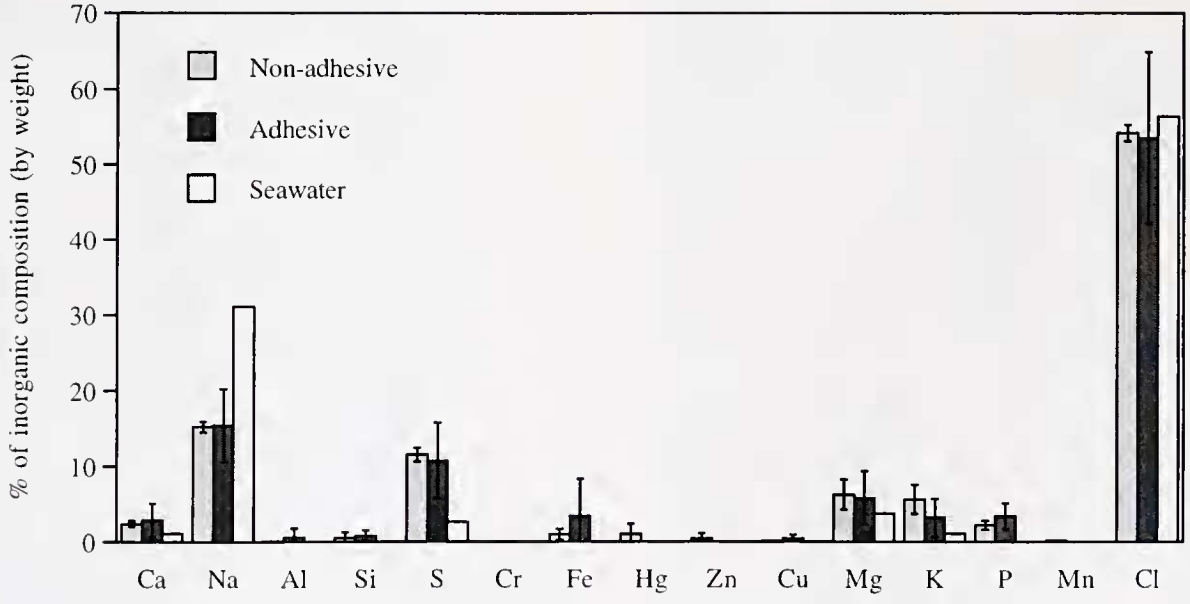

Figure 2. Scanning electron microscope elemental analysis of non-adhesive and adhesive mucus. Data for seawater are adapted from Schmidt-Nielsen (1990) and are included for comparison. The relative contribution of each element to the total weight of inorganic matter is shown.

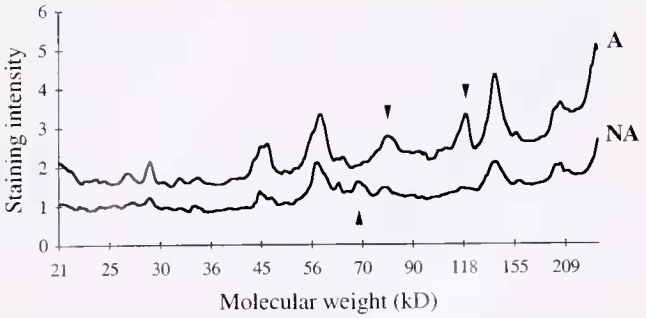

Figure 3. Average staining intensity of the bands resulting from SDS-PAGE performed on adhesive (A) and non-adhesive (NA) mucus. The relative staining intensity is measured by the absorbance at 580 nm. The 118 kD, 80 kD, and 68 kD proteins are marked by arrowheads.

Comparison of protein composition

SDS-PAGE showed that, with a few significant exceptions, the same proteins were present in the adhesive and non-adhesive forms of mucus (Figs. 3, 4). The most striking difference was a 118 kD protein that was common in the adhesive form, but rare in the non-adhesive form. In addition, an 80 kD protein was more common in the adhesive form; but it was not consistently present. Conversely, a 68 kD protein was usually found in the non-adhesive mucus, though in relatively smaller quantities, and it was not found in the adhesive mucus.

There was some variability in the amount of many of the proteins. In Figure 4, for example, the 57 kD protein appeared to be more common in the non-adhesive mucus, but this was not a consistent difference. In some samples, this protein was more common in the adhesive mucus instead. In addition, the 80 kD protein was sometimes quite a bit darker than shown in Figure 4. Thus, it was important to average a number of samples to identify consistent differences.

In addition to the consistent difference in a few specific proteins, the SDS-PAGE results also supported the overall difference in protein concentration. On average, the intensity of staining of lanes containing adhesive mucus was 1.8 times greater than that of lanes containing non-adhesive mucus. Adjusting for the slight difference in the amount of mucus loaded gave a figure of 2.0 times as much protein in the adhesive mucus.

Analysis of samples run on gels with different pore sizes showed that the proteins shown in Figures 3 and 4 make up the bulk of the secretion. Lower and higher percentage gels showed no significant proteins above 220 kD or below 20 kD. There was, however, often material that failed to enter the stacking gel, forming a band at the top. Using the densitometer, it was calculated that this amounted to roughly 10% of the total protein. Several lines of evidence suggested that this band was due to aggregation of the other proteins (see Discussion). In any case, there was no difference in the staining intensity of this band between the two forms of mucus.

Solubility

The mucus was difficult to solubilize, but certain conditions improved its solubility. Solubility was improved in basic rather than acidic conditions. Urea, SDS, and reducing agents improved the solubility, but each of these on their own extracted only one-quarter to one-half of the protein extractable by all three together. Moreover, buffers that increased extraction did so by extracting more of the same proteins; *i.e.*, the banding pattern in SDS-PAGE did not change. The effect of reducing agents, however, was exceptional: gels run without reducing agents were more prone to defects and had bands that were less distinct, so these gels were harder to read. Some proteins appeared to be poorly extracted in the absence of reducing agents, but this difference was not clear cut.

The effect of shear during extraction was similar to the effect of stronger buffers. Stronger shear gave greater ex-

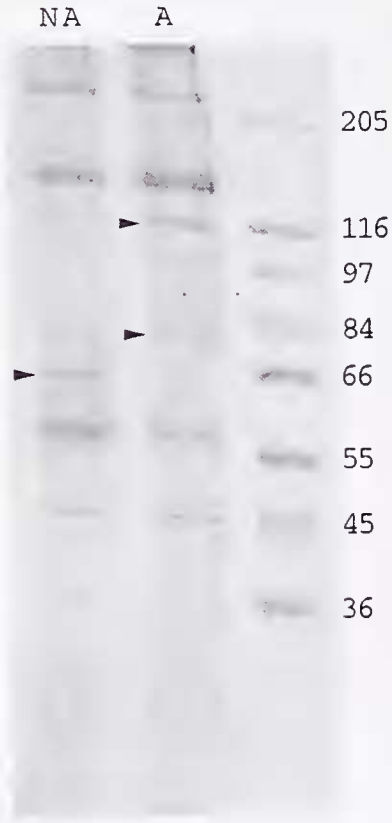

Figure 4. Example of an SDS-PAGE comparison of a non-adhesive (NA) and an adhesive (A) mucus sample (7 and 10 μg protein loaded respectively). The gel was stained with Coomassie blue R-250. Molecular weight markers are in the right lane and have the following molecular weights: 205, 116, 97, 84, 66, 55, 45 and 36 kD. The 118 kD, 80 kD, and 68 kD proteins are marked by arrowheads.

Table I

Amino acid compositions of selected proteins found in the pedal mucus of the limpet Lottia limatula *(values in residues per thousand)*

	45 kD	57 kD	68 kD	118 kD	140 kD	Whole mucus (adhesive)
ASX	96 (95)	117 (102)	138 (104)	142 (118)	154 (129)	127
THR	52 (51)	63 (54)	57 (47)	72 (60)	76 (63)	116
SER	66 (71)	74 (88)	120 (114)	97 (105)	49 (69)	90
GLX	125 (124)	134 (122)	98 (101)	144 (130)	140 (128)	115
PRO	46 (45)	37 (38)	42 (41)	48 (46)	113 (89)	79
GLY	133 (141)	89 (121)	108 (144)	79 (122)	80 (110)	80
ALA	72 (71)	77 (75)	97 (83)	55 (64)	55 (61)	52
VAL	53 (49)	40 (59)	0 (42)	22 (52)	32 (51)	72
MET	27 (25)	44 (27)	39 (18)	21 (13)	25 (17)	11
ILEU	55 (53)	42 (45)	34 (44)	51 (37)	45 (47)	52
LEU	73 (72)	65 (67)	76 (73)	51 (61)	49 (57)	51
TYR	31 (35)	39 (35)	34 (32)	26 (18)	30 (30)	18
PHE	39 (35)	55 (41)	56 (35)	39 (31)	38 (32)	26
HIS	22 (23)	32 (32)	17 (26)	28 (31)	26 (28)	10
LYS	57 (57)	51 (47)	66 (51)	90 (70)	58 (52)	59
ARG	52 (52)	46 (46)	41 (44)	30 (38)	32 (37)	33
CYS/2	—	—	—	4 (2)	—	20

For each amino acid, the first value is corrected for the blank; values in parentheses are unblanked. Note that cysteine was not quantified, only the acid-stable, disulfide-bonded form CYS/2. These bonds were reduced in the individual proteins, but not the whole mucus sample.

traction, but SDS-PAGE showed that exactly the same proteins were present in the same proportions as in samples dissolved with mild shear. This is significant because Sajdera and Hascall (1969) showed that sonication can fracture the large molecules that make up mammalian mucous gels. This did not appear to be the case with limpet mucus.

The adhesive mucus was typically more difficult to dissolve than the non-adhesive mucus. It often took twice as long to break up visibly during any extraction. In addition, it was much less soluble in neutral salt buffers than the non-adhesive form.

Characterization of proteins

Most of the major proteins were acidic. The 118 kD protein had an isoelectric point between 5 and 5.3. The 203, 140, 57, and 45 kD proteins had isoelectric points ranging from 4.7 to 5.3. The 80 and 68 kD proteins appeared to fall within a similar range of isoelectric points, but they did not show up clearly enough to be identified unequivocally. The only significant proteins that were not so acidic were a 29 kD protein at a pI of 6.5, and a 53 kD protein at a pI of about 8.6.

Of the proteins identified by electrophoresis, only the 140 kD protein was a glycoprotein. This finding was based on glycan staining of adhesive and non-adhesive mucus samples that were run on SDS-PAGE and transferred to nitrocellulose. Transient staining with Ponceau-S showed that all the proteins transferred successfully. The glycan detection kit stained the 140 kD protein darkly in the adhesive and non-adhesive samples. No other protein was marked by this stain. The blot showed a fair amount of background staining at the top of both samples, at about 100 kD and above.

The proteins that were analyzed had similar amino acid compositions (Table I). The method of Marchalonis and Weltman (1971) gave relatedness values ($S\Delta Q$) ranging from 54 to 101 for the comparison between the 118 kD protein and the other proteins in Table I; any value lower than 100 suggests relatedness. All of the proteins had many polar or charged amino acids, especially acidic ones. The 118 kD protein had the largest proportion of these, with 65% of its amino acids either electrically charged at physiological pH, or polar. Finally, the amino acid compositions of the identified proteins were similar to the composition of whole adhesive mucus. This supports the finding that they make up the bulk of the secretion. Note that our hydrolysis conditions did not allow us to quantify cysteine. In addition, due to high backgrounds, slow transfer of some of the proteins, and small initial quantities, the correction based on the blank was significant and may have led to some errors, especially for the 68 kD protein.

Though most of the proteins transferred easily, the 118, 80, and 53 kD proteins transferred several times more slowly in the Tris-borate buffer, despite the presence of SDS. This finding was based on quantitative comparison of stained pre-transfer and post-transfer lanes and blots, adjusting for the effect of molecular weight. The slow transfer of the 80 kD protein, combined with its small initial quantities and position near several minor bands that transferred

well, made it difficult to identify unequivocally on the membrane. Thus, its amino acid composition was not analyzed.

Discussion

Adhesive limpet mucus differs from non-adhesive limpet mucus in three clear ways: first, an overall twofold increase in protein and carbohydrate concentration; second, the addition of a 118 kD protein; third, the absence of a 68 kD protein. Another difference is the presence of an 80 kD protein in adhesive mucus, though this was not consistently present. There may be similar differences in specific carbohydrates, but this study did not analyze carbohydrate composition in detail. Therefore, although we have identified clear biochemical differences, these may not be the only differences between the two forms of mucus. Nevertheless, the identified changes could account for much of the difference in function.

To understand the possible effects of these changes, we will first review what is known of the structure of mucous gels, and how limpet pedal mucus compares to other mucous gels. Then we can consider the factors that affect mucous gel mechanics. This will give insight into the significance of the biochemical changes identified in this paper. With this understanding, we can consider limpet adhesion in the context of other invertebrate adhesives.

Structure of mucous gels

Mucous gels are typically made of poly-anionic polymers that take up an expanded configuration due to electrostatic repulsion (Wainwright *et al.*, 1976). Mammalian mucus, the best studied mucous gel, is based on the mucin glycoprotein. Mucin is enormous; gastric mucin is composed of four disulfide-linked subunits of over 500 kD each, resulting in a molecule of about 2000 kD (Allen, 1977; Allen *et al.*, 1984). Other mucins may be much larger (Silberberg, 1989). These molecules are heavily glycosylated (Jentoft, 1990), typically consisting of 70%–80% carbohydrate (Hafez, 1977). Hundreds of short carbohydrate chains are attached to serine and threonine residues in the glycosylated region (Silberberg and Meyer, 1982). Thus, serine and threonine typically make up 30% of the amino acids in mucin (Pearson *et al.*, 1982; Allen *et al.*, 1984; Smith and Lamont, 1984). The carbohydrates carry a substantial negative charge, which causes the molecule to take on an extended configuration and fill a large volume. When the mucin concentration reaches 2% to 4%, the molecules interact and become entangled, forming a viscoelastic gel (Allen, 1977).

Limpet pedal mucus also forms gels from a protein-polysaccharide mixture at similar concentrations. Nevertheless, it appears to be based on a different type of molecule. Carbohydrates make up only about 15% of the organic material, and only one of the proteins is glycosylated. The amino acid compositions of the proteins in limpet mucus are also different from mucin; for example, in the identified proteins, serine and threonine make up 12% to 18% of the total residues, rather than 30%. A comparison of the amino acid composition of vertebrate mucin (Smith and Lamont, 1984) with that of the 140 kD glycoprotein of limpet pedal mucus gives an $S\Delta Q$ value of 342. This strongly suggests that the proteins are not related.

Limpet mucus appears to be made of smaller subunits than vertebrate mucus. The bulk of the material was composed of proteins ranging from 20 to 220 kD. There was a component that was too big to enter the stacking gel, but it represented only about 10% of the protein, and may have been due to aggregation of the other proteins. The gels showed characteristics typical of aggregation at the top, such as streaking and high background staining in the lanes (Hames, 1990). Also, other experiments have suggested that the proteins in limpet mucus have a strong tendency to aggregate; in gel filtration columns using gel media that should achieve some separation of these proteins, the proteins tended to elute together, even when urea or guanidine hydrochloride was added to the buffer (personal communication).

Limpet pedal mucus also appears to be stronger than mammalian mucus. Mammalian tracheal mucin typically has a dynamic viscosity of 1 to 1000 poise. Its storage and loss moduli, which are measures of elasticity and viscosity, are typically 1 to 10 Pa, depending on the strain rate (Litt *et al.*, 1977). Other mammalian mucous secretions have similar values. In contrast, Grenon and Walker (1980) found the viscosity of pedal mucus from the limpet *Patella vulgata* to be on the order of 10^6 to 10^7 poise, and the elastic modulus to be between 300 and 7000 Pa. These were preliminary measurements derived from a creep test rather than a dynamic tester, so they are not strictly comparable; moreover, the four samples tested by Grenon and Walker varied considerably. Nevertheless, these data indicate a substantial difference in mechanical properties from vertebrate mucus. Such a difference was also apparent when solubilizing the mucus. Mammalian mucin dissolves easily in 6 *M* GuCl (Allen, 1977) or 0.2 *M* 2-mercaptoethanol (Allen *et al.*, 1984). Limpet mucus does not dissolve well, especially without strong shearing. This is not surprising, since a glue must be insoluble to function effectively.

Though limpet mucus differs from mammalian mucus in biochemistry and strength, it probably forms gels in the same way. Presumably, the proteins and polysaccharides are linked together into a larger network. They may form large complexes that entangle, or they may form a fully cross-linked network. The effect of SDS, urea, and reducing agents on the solubility of limpet mucus suggests that disulfide bonds and a variety of non-covalent bonds are important for linking these subunits together.

Factors that affect the mechanics of polymer gels and their relation to adhesion

Polymer gels are dominated by the effects of entanglement between large macromolecules. To deform such a gel, the polymers must be pulled apart. At short time intervals, the "knots" between polymers cannot be undone, and the gel behaves elastically. Over longer time periods, though, the polymers can creep and work out of their local entanglements, effectively slipping through the boundaries imposed by their neighboring molecules. This process is called reptation (deGennes and Leger, 1982).

A variety of factors affect the mechanics of polymer gels (Doi and Edwards, 1988). Clearly the polymer concentration will be critical. As the concentration increases, each polymer interacts with a larger number of others, further impeding its ability to creep. Characteristics of the polymers also affect the mechanics of the gel. Longer polymers entangle more easily and have a harder time pulling out of these entanglements. Any branching of the polymers dramatically slows their ability to pull free of one another, as does any other interaction between polymers.

Given these considerations, what is the significance of the identified changes in limpet pedal mucus? The twofold increase in concentration certainly would increase the stiffness and viscosity of the gel. Silberberg and Meyer (1982) argue that, in practice, the polymer concentration may be the most important factor regulating the mechanics of mucous gels. This has been demonstrated for mammalian cervical mucus (Tam and Verdugo, 1981).

Though the effect of increased concentration is clear, the cause of the increase is unknown. It is probably not as simple as secreting a higher concentration of protein, since a gel will swell or shrink to reach an equilibrium volume determined by a variety of factors. These include the rubbery elasticity of the polymers, the osmotic pressure of ions trapped in the polyanionic network, and the interactions between polymer and solvent (Tanaka, 1981). Change in any of these factors can cause shrinkage or swelling of the gel, thus effectively changing the polymer concentration. For example, salt concentration and pH both affect the swelling pressure of the gel. These factors could be controlled by the secreting epithelium (Tam and Verdugo, 1981, 1982). A large change in conditions may not even be necessary; if the conditions are right, a small change in one factor may trigger a phase change in the gel from swollen to collapsed (Tanaka, 1981), which could easily produce a twofold increase in concentration. The addition of a protein containing large numbers of basic residues could trigger such a collapse. For this reason, it is suggestive that the 118 kD protein contains significantly more basic amino acids than the other proteins in the secretion.

Though the concentration of the gel clearly affects its mechanics, the significance of the 118 kD protein in adhesion is less certain. It probably plays a central role, however, since the total polymer concentration of adhesive limpet mucus is similar to that of mammalian mucus, yet the former is dramatically more effective as an adhesive. It seems that there must be a change in addition to the concentration increase to account for the adhesiveness of limpet mucus. Perhaps the 118 kD protein links gel-forming polymers into larger functional molecules, possibly introducing branching. If so, it would strengthen the entangling interactions, and might even lead to the formation of a fully cross-linked network. Otani *et al.* (1996) show that hydrogels composed of gelatin and poly(L-glutamic acid) can increase in adhesiveness roughly a hundred-fold with the addition of a cross-linking agent. Incidentally, gelatin units of 20 to 60 kD form gels at a concentration just above 4.4% (Otani *et al.*, 1996), thus a molecule need not be enormous to form effective gels.

The 68 kD and 80 kD proteins were not as consistently linked to the change in adhesiveness as the 118 kD protein, but it is likely that they also have roles. The 80 kD protein may have a function similar to that of the 118 kD protein. The 68 kD protein, on the other hand, may release the adhesion by breaking some of the connections between gel-forming molecules; it could also be a breakdown product of one of these proteins. Alternatively, it could be a different type of gel-forming molecule that forms weaker links.

The inorganic composition does not appear to be related to the adhesiveness of limpet mucus. It has been suggested that divalent ions stiffen mucus by forming electrovalent cross-links (Hermans, 1983; Thomas and Hermans, 1985). Marriott *et al.* (1982) showed that increasing the calcium concentration from 0 to 30 m*M* can roughly triple the viscosity of mucus. Multivalent ions in general have a prominent effect on polyanionic gels (Tanaka, 1981). Nevertheless, there were no differences in the relative proportions of ions such as calcium and magnesium between adhesive and non-adhesive mucus.

Significance for invertebrate adhesion

In contrast to mucus-based adhesives, solid adhesives have been studied more thoroughly. Barnacles adhere using a proteinaceous cement that includes relatively little water (see Walker, 1972; Barnes and Blackstock, 1974; Walker and Youngson, 1975; Yule and Walker, 1987; Naldrett, 1993; Kamino *et al.*, 1996). As with limpets, disulfide bonds and non-covalent linkages appear important for linking the barnacle's adhesive proteins (Naldrett, 1993; Kamino *et al.*, 1996). Mussels adhere using proteinaceous byssal threads and adhesive plaques (see Waite and Tanzer, 1981; Waite, 1983, 1985; Waite *et al.*, 1989; Rzepecki *et al.*, 1992; Papov *et al.*, 1995). These are typically cross-linked using a quinone-tanning process that relies on the presence of the

amino acid DOPA (Waite and Tanzer, 1981). Also, certain tube-dwelling worms secrete a proteinaceous cement containing DOPA (Jensen and Morse, 1988). A fundamental difference between these adhesives and limpet pedal mucus is that mucus is 90% to 95% water and therefore has much lower tensile stiffness. Thus, the mechanism by which it forms strong attachments has been poorly understood.

Some studies have suggested that mucus is inherently adhesive due to its viscoelasticity (Grenon and Walker, 1981). This is unlikely to be true; as pointed out in the Introduction, mucus is more often used as a lubricant. Despite their viscoelasticity, typical mucus secretions are not sticky.

It has also been suggested that mucus forms bonds through Stefan adhesion, which occurs when a high-viscosity secretion is trapped between two closely apposed, rigid plates. Because of its viscosity, the secretion resists the flow that must accompany the separation of the plates (Grenon and Walker, 1981). However, Stefan adhesion produces much weaker bonds than the Stefan equation predicts for two reasons. First, it depends on having rigid adherends. Any flexibility in the adherends dramatically reduces the adhesive force. Thus, the Stefan equation is not relevant to soft-bodied invertebrates. Second, even if the adherends were rigid enough, Stefan adhesion is limited because cavitation of water in the mucus would typically cause failure at tenacities of roughly 50 kPa (Banks and Mill, 1953). This is considerably weaker than the tenacities of most limpets using glue-like adhesion. Furthermore, Stefan adhesion provides little shear resistance, yet greatly increased shear resistance is one of the defining characteristics of the change from suction to glue-like adhesion (Smith, 1992). Smith (1991b) provides further evidence that limpets, in particular, do not use Stefan adhesion.

Although many mucous secretions are not normally adhesive, the present study shows that they can be modified to become adhesive. This is particularly interesting given the wide variety of animals that use mucus-based secretions for adhesion. Echinoderm tube feet adhere using a viscous secretion typically identified as mucus (Chaet and Philpott, 1964; Harrison, 1966; Souza Santos, 1966; Thomas and Hermans, 1985; Ball and Jangoux, 1990; Flammang *et al.*, 1991; Flammang and Jangoux, 1992, 1993). Various holothurians capture food on their tentacles with a viscous mucus or proteinaceous secretion (Cameron and Fankboner, 1984; McKenzie, 1987). In many of these studies, mucus is defined as a viscous secretion containing protein and mucopolysaccharides. Brown algae also adhere with a mucopolysaccharide or glycoprotein (Oliveira *et al.*, 1980). Many turbellarians and gastrotrichs also produce viscid adhesive secretions, and possibly releasing secretions, some of which may involve mucus (Tyler, 1976; Tyler and Rieger, 1980). Barnacle larvae apparently produce a viscous, proteinaceous adhesive secretion (Walker and Yule, 1984).

What makes these secretions adhesive instead of slippery? Many of the studies cited demonstrate the presence of more than one type of secretory cell on the adhesive epithelium, each capable of adding a different component to the secretion. The difficulty is the relative lack of direct evidence that identifies which components are responsible for adhesion. Most of the evidence is based on the locations and arrangements of the different cells. In some cases, mucopolysaccharides are believed to be adhesive, and in others the protein components are believed to be adhesive. Hermans (1983) suggested that many adhesive organs produce two separate secretions: an adhesive secretion that may be proteinaceous and a releasing or de-adhesive secretion based on mucopolysaccharides. The present study provides a related possibility—one fundamental secretion that can be modified to be either adhesive or non-adhesive. The type of changes identified in limpet pedal mucus may provide a paradigm that will be useful in understanding the adhesion of other mucus-secreting animals.

Acknowledgments

We thank J. H. Waite for performing amino acid analyses and R. Walson for his assistance with the scanning electron microscopy. We thank two anonymous reviewers for their thoughtful comments. This research was supported by a grant from the Holcomb Research Institute to A.M.S., and student research awards to T.J.Q and R.L.S. from the Holcomb Research Institute.

Literature Cited

Allen, A. 1977. Structure and function in gastric mucus. In *Mucus in Health and Disease*, M. Elstein and D. Parke, eds. *Adv. Exp. Med. Biol.* **89:** 283–299. Plenum Press, New York.

Allen, A., D. A. Hutton, J. P. Pearson, and L. A. Sellers. 1984. Mucus glycoprotein structure, gel formation and gastrointestinal mucus function. In *Mucus and Mucosa*, J. Nugent and M. O'Connor, eds. *Ciba Found. Symp.* **109:** 137–156. Pitman, London.

Baier, R. E., H. Gucinski, M. A. Meenaghan, J. Wirth, and P. O. Glantz. 1985. Biophysical studies of mucosal surfaces. Pp. 83–95 in *Oral Interfacial Reactions of Bone, Soft Tissue and Saliva*, P. O. Glantz, S. A. Leach, and T. Ericson, eds. IRL Press, Oxford.

Ball, B., and M. Jangoux. 1990. Ultrastructure of the tube foot sensory-secretory complex in *Ophiocomina nigra* (Echinodermata, Ophiuridae). *Zoomorphology* **109:** 201–209.

Banks, W. H., and C. C. Mill. 1953. Tacky adhesion—a preliminary study. *J. Colloid Sci.* **8:** 137–147.

Barnes, H., and J. Blackstock. 1974. The biochemical composition of the cement of a pedunculate cirripede. *J. Exp. Mar. Biol. Ecol.* **16:** 87–91.

Boyer, R. F. 1993. *Modern Experimental Biochemistry.* Benjamin/Cummings, Redwood City, CA. 555 pp.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.

Branch, G. M., and A. C. Marsh. 1978. Tenacity and shell shape in six

Patella species: adaptive features. *J. Exp. Mar. Biol. Ecol.* **34:** 111–130.

Cameron, J. L., and P. V. Fankboner. 1984. Tentacle structure and feeding processes in life stages of the commercial sea cucumber *Parastichopus californicus* (Stimpson). *J. Exp. Mar. Biol. Ecol.* **81:** 193–209.

Chaet, A. B., and D. E. Philpott. 1964. A new subcellular particle secreted by the starfish. *J. Ultrastruct. Res.* **11:** 354–362.

Chantler, E., and E. Debruyne. 1977. Factors regulating the changes in cervical mucus in different hormonal states. In *Mucus in Health and Disease,* M. Elstein and D. Parke, eds. *Adv. Exp. Med. Biol.* **89:** 131–141. Plenum Press, New York.

Davies, M. S., H. D. Jones, and S. J. Hawkins. 1990. Seasonal variation in the composition of pedal mucus from *Patella vulgata* L. *J. Exp. Mar. Biol. Ecol.* **144:** 101–112.

deGennes, P. G., and L. Leger. 1982. Dynamics of entangled polymer gels. *Annu. Rev. Phys. Chem.* **33:** 49–61.

Denny, M. W. 1980. The role of gastropod pedal mucus in locomotion. *Nature* **285:** 160–161.

Denny, M. W. 1989. Invertebrate mucous secretions: functional alternatives to vertebrate paradigms. In *Mucus and Related Topics,* E. Chantler and N. A. Ratcliffe, eds. *Symp. Soc. Exp. Biol.* **43:** 337–366. Society for Experimental Biology, Cambridge.

Doi, M., and S. F. Edwards. 1988. *The Theory of Polymer Dynamics.* Clarendon Press, Oxford. 391 pp.

Flammang, P., and M. Jangoux. 1992. Functional morphology of the locomotory podia of *Holothuria forskali* (Echinodermata, Holothuroida). *Zoomorphology* **111:** 167–178.

Flammang, P., and M. Jangoux. 1993. Functional morphology of coronal and peristomeal podia in *Sphaerechinus granularis* (Echinodermata, Echinoida). *Zoomorphology* **113:** 47–60.

Flammang, P., C. DeRidder, and M. Jangoux. 1991. Ultrastructure of the penicillate podia of the spatangoid echinoid *Echinocardium cordatum* (Echinodermata) with special emphasis on the epidermal sensory-secretory complexes. *Acta Zool. (Stockh.)* **72:** 151–158.

Grenon, J. F., and G. Walker. 1978. The histology and histochemistry of the pedal glandular system of two limpets, *Patella vulgata* and *Acmaea tessulata* (Gastropoda: Prosobranchia). *J. Mar. Biol. Ass. UK* **58:** 803–816.

Grenon, J. F., and G. Walker. 1980. Biomechanical and rheological properties of the pedal mucus of the limpet, *Patella vulgata* L. *Comp. Biochem. Physiol. B.* **66:** 451–458.

Grenon, J. F., and G. Walker. 1981. The tenacity of the limpet, *Patella vulgata* L.: an experimental approach. *J. Exp. Mar. Biol. Ecol.* **54:** 277–308.

Hafez, E. S. E. 1977. Functional anatomy of mucus-secreting cells. In *Mucus in Health and Disease,* M. Elstein and D. Parke, eds. *Adv. Exp. Med. Biol.* **89:** 19–38. Plenum Press, New York.

Hames, B. D. 1990. One-dimensional polyacrylamide gel electrophoresis. Pp. 1–147 in *Gel Electrophoresis of Proteins: A Practical Approach,* B. D. Hames and D. Rickwood, eds. IRL Press, Oxford.

Harrison, G. A. 1966. Subcellular particles in echinoderm tube feet. I. Class Asteroidea. *J. Ultrastruct. Res.* **16:** 537–547.

Harrold, C. 1982. Escape responses and prey availability in a kelp forest predator-prey system. *Am. Nat.* **119:** 132–135.

Hermans, C. O. 1983. The duo-gland adhesive system. *Oceanogr. Mar. Biol. Annu. Rev.* **21:** 283–339.

Jensen, R. A., and D. E. Morse. 1988. The bioadhesive of *Phragmatopoma californica* tubes—a silk-like cement containing L-Dopa. *J. Comp. Phy. B.* **158:** 317–324.

Jentoft, N. 1990. Why are proteins O-glycosylated? *Trends Biochem. Sci.* **15:** 291–294.

Kamino, K., S. Odo, and T. Maruyama. 1996. Cement proteins of the acorn barnacle, *Megabalanus rosa. Biol. Bull.* **190:** 403–409.

Kennedy, J. F., and G. Pagliuca. 1994. Oligosaccharides. Pp. 43–72 in *Carbohydrate Analysis: A Practical Approach,* M. F. Chaplin and J. F. Kennedy, eds. IRL Press, Oxford.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.

Litt, M., D. P. Wolf, and M. A. Khan. 1977. Functional aspects of mucus rheology. In *Mucus in Health and Disease,* M. Elstein and D. Parke, eds. *Adv. Exp. Med. Biol.* **89:** 191–201. Plenum Press, New York.

Marchalonis, J. J., and J. K. Weltman. 1971. Relatedness among proteins: a new method of estimation and its application to immunoglobins. *Comp. Biochem. Physiol.* **38B:** 609–625.

Marriott, C., M. F. Beeson, and D. T. Brown. 1982. Biopolymer induced changes in mucus viscoelasticity. In *Mucus in Health and Disease II,* E. N. Chantler, J. B. Elder, and M. Elstein, eds. *Adv. Exp. Med. Biol.* **144:** 89–92. Plenum Press, New York.

McKenzie, J. D. 1987. The ultrastructure of the tentacles of 11 species of dendrochirote holothurians studied with special reference to the surface coats and papillae. *Cell Tissue Res.* **248:** 187–199.

Naldrett, M. J. 1993. The importance of sulphur cross-links and hydrophobic interactions in the polymerization of barnacle cement. *J. Mar. Biol. Ass. UK* **73:** 689–702.

Oliveira, L., D. C. Walker, and T. Bisapultra. 1980. Ultrastructural, cytochemical and enzymatic studies on the adhesive plaques of brown algae *Laminaria saccharina* (L.) and *Nereocystis luckeana* (Nert.). *Protoplasma* **104:** 1–15.

Otani, Y., Y. Tabata, and Y. Ikada. 1996. Rapidly curable biological glue composed of gelatin and poly(L-glutamic acid). *Biomaterials* **17:** 1387–1391.

Papov, V. V., T. V. Diamond, K. Biemann, and J. H. Waite. 1995. Hydroxyarginine-containing polyphenolic proteins in the adhesive plaques of the marine mussel *Mytilus edulis. J. Biol. Chem.* **270:** 20183–20192.

Pearson, J. P., R. Kaura, W. Taylor, and A. Allen. 1982. The composition and polymeric structure of mucus glycoprotein from human gallbladder bile. *Biochim. Biophys. Acta* **706:** 221–228.

Rzepecki, L. M., K. M. Hansen, and J. H. Waite. 1992. Characterization of a cystine-rich polyphenolic protein family from the blue mussel *Mytilus edulis* L. *Biol. Bull.* **183:** 123–137.

Sajdera, S. W., and V. C. Hascall. 1969. Proteinpolysaccharide complex from bovine nasal cartilage. *J. Biol. Chem.* **244:** 77–87.

Schmidt-Nielsen, K. 1990. *Animal Physiology: Adaptation and Environment.* Cambridge University Press, Cambridge. 607 pp.

Silberberg, A. 1989. Mucus glycoprotein, its biophysical and gel-forming properties. In *Mucus and Related Topics,* E. Chantler and N. A. Ratcliffe, eds. *Symp. Soc. Exp. Biol.* **43:** 43–63. Society for Experimental Biology, Cambridge.

Silberberg, A., and F. A. Meyer. 1982. Structure and function of mucus. In *Mucus in Health and Disease II,* E. N. Chantler, J. B. Elder, and M. Elstein, eds. *Adv. Exp. Med. Biol.* **144:** 53–74. Plenum Press, New York.

Smith, A. M. 1991a. Negative pressure generated by octopus suckers: a study of the tensile strength of water in nature. *J. Exp. Biol.* **157:** 257–271.

Smith, A. M. 1991b. The role of suction in the adhesion of limpets. *J. Exp. Biol.* **161:** 151–169.

Smith, A. M. 1992. Alternation between attachment mechanisms by limpets in the field. *J. Exp. Mar. Biol. Ecol.* **160:** 205–220.

Smith, A. M. 1996. Cephalopod sucker design and the physical limits to negative pressure. *J. Exp. Biol.* **199:** 949–958.

Smith, A. M., W. M. Kier, and S. Johnsen. 1993. The effect of depth on the attachment force of limpets. *Biol. Bull.* **184:** 338–341.

Smith, B. F., and J. T. LaMont. 1984. Hydrophobic binding properties of bovine gallbladder mucin. *J. Biol. Chem.* **259:** 12170–12177.

Souza Santos, H. D. 1966. The ultrastructure of the mucous granules from starfish tube feet. *J. Ultrastruct. Res.* **16:** 259–268.

Tam, P. Y., and P. Verdugo. 1981. Control of mucus hydration as a Donnan equilibrium process. *Nature* **292:** 340–342.

Tam, P. Y., and P. Verdugo. 1982. Mucus hydration: a Donnan equilibrium controlled process. In *Mucus in Health and Disease II,* E. N. Chantler, J. B. Elder, and M. Elstein, eds. *Adv. Exp. Med. Biol.* **144:** 101–103. Plenum Press, New York.

Tanaka, T. 1981. Gels. *Sci. Am.* **244** (January): 124–138.

Thomas, L. A., and C. O. Hermans. 1985. Adhesive interactions between the tube feet of a starfish, *Leptasterias hexactis,* and substrata. *Biol. Bull.* **169:** 675–688.

Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76:** 4350–4354.

Tyler, S. 1976. Comparative ultrastructure of adhesive systems in the Turbellaria. *Zoomorphologie* **84:** 1–76.

Tyler, S., and G. E. Rieger. 1980. Adhesive organs of the Gastrotricha I. Duo-gland organs. *Zoomorphologie* **95:** 1–15.

Wainwright, S. A., W. D. Biggs, J. D. Currey, and J. M. Gosline. 1976. *Mechanical Design in Organisms.* Princeton University Press, Princeton. 423 pp.

Waite, J. H. 1983. Adhesion in bysally attached bivalves. *Biol. Rev. Camb. Philos. Soc.* **58:** 209–231.

Waite, J. H. 1985. Peptide repeats in a mussel glue protein: theme and variations. *Biochemistry* **24:** 5010–5014.

Waite, J. H., and M. L. Tanzer. 1981. Polyphenolic substance of *Mytilus edulis:* novel adhesive containing L-Dopa and hydroxyproline. *Science* **212:** 1038–1040.

Waite, J. H., D. C. Hansen, and K. T. Little. 1989. The glue protein of ribbed mussels (*Geukensia demissa*): a natural adhesive with some features of collagen. *J. Comp. Physiol. B.* **159:** 517–525.

Walker, G. 1972. The biochemical composition of the cement of two barnacle species, *Balanus hameri* and *Balanus crenatus. J. Mar. Biol. Ass. UK* **52:** 429–435.

Walker, G., and A. Youngson. 1975. The biochemical composition of *Lepas anatifera* (L.) cement (Crustacea: cirripedia). *J. Mar. Biol. Ass. UK* **55:** 703–707.

Walker, G., and A. B. Yule. 1984. Temporary adhesion of the barnacle cyprid: the existence of an antennular adhesive secretion. *J. Mar. Biol. Ass. UK* **64:** 679–686.

Yule, A. B., and G. Walker. 1987. Adhesion in barnacles. Pp. 389–402 in *Crustacean Issues: Barnacle Biology,* Vol. 5, A. J. Southward, ed. Balkema, Rotterdam.